Remarks

The Office Action mailed November 25, 2009, has been received and reviewed. Claims 1-4, 6-9 and 11-13 are pending. Of these, claims 3, 4, 7 and 12 have been withdrawn from consideration by the Examiner as drawn to a noneleted invention. Accordingly, claims 1, 2, 6, 8, 9, 11 and 13 are currently under examination. Claims 1, 9, and 13 have been amended. Reconsideration and withdrawal of the rejections are respectfully requested.

Support for the amendments to the specification at page 4, lines 24 through 27 reciting "Δ309-489" in the figure legends for Figs. 2 and 3 is found in Figure 2 and Figure 3 as originally filed and also in the specification at, for example, page 19, lines 11-12, and page 19, lines 17-21.

Rejection under 35 U.S.C. §112, First Paragraph

Claims 1, 2, 6, 8, 9, 11, and 13, were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Office states that the recitation of "amino acids 309 to 489 in SEQ ID NO:1" does not correspond to the nucleic acid of SEQ ID NO:1.

Claims 1, 9 and 11 have been amended to recite "an amino acid sequence encoded by codons 309 to 489" thereby obviating the rejection.

Reconsideration and withdrawal of the rejection of claims 1, 2, 6, 8, 9, 11, and 13, under 35 U.S.C. §112, second paragraph, are respectfully requested.

Rejection under 35 U.S.C. §112, First Paragraph

The rejection of claims 1, 8, and 13 under 35 U.S.C. 112, first paragraph, as failing to comply with written description requirement, is maintained. Specifically, the Office states that the specification has not provided adequate description indicating which are claimed subgenus retroviruses pseudotyped with modified GPs that have transduction efficiency at least 2-fold higher than those with the wild-type GP in a genus of retroviruses pseudotyped with any

modified GPs in the O-glycosylation region, referring back to paragraphs 12-16 in the Office Action mailed April 1, 2009. Applicants respectfully traverse this rejection.

Paragraph 12 of the earlier Office Action mailed April 1, 2009, cites the recitation in claim 1 of "a glycoprotein comprising a modified O-glycosylation **region**" (emphasis in the original) and asserts that neither the specification nor the claims define this phrase. As a result, the Office Action asserted that "the scope of the claims encompasses a genus of retroviruses pseudotyped with *any* GP that contains *any* modification" (emphasis added).

Although traversing the rejection, Applicants did amend claim 1 to delete recitation of "a glycoprotein comprising a modified O-glycosylation region" and recite in its place "an Ebola glycoprotein containing a deletion of amino acids [amended herewith to read "an amino acid sequence encoded by codons"] 309-489 of SEQ ID NO:1 in the *O*-glycosylation region." Applicants submit that this amendment was fully responsive to the rejection. As to *which* glycoprotein, claim 1 was amended to recite an Ebola glycoprotein. As to *which* modification, claim 1 was amended to recite deletion of amino acids [codons] 309-489 of SEQ ID NO:1.

Applicants respectfully disagree with the statement at paragraph 13 of the Office Action mailed April 1, 2009, that the specification has disclosed only one species of the claimed genus of pseudotyped retroviruses. To the contrary, the specification contains working examples that describe at least *two distinct species* of pseudotyped retrovirus having a retroviral core and an Ebola glycoprotein containing a deletion of codons 309 to 489 in SEQ ID NO:1.

First, as noted by the Office, Example I, beginning on page 16, discloses a pseudotyped retrovirus with a MuLV core and an Ebola glycoprotein containing a deletion of codons 309 to 489 in SEQ ID NO:1. A pseudotyped retrovirus with MuLV core and control elements having "a corresponding increase of 696 ±142% in transduction by the Δ309-489 GP pseudotyped viruses as compared to wild type" is described in the specification at page 19, lines 17-19.

In addition, Example III, beginning on page 21, discloses a pseudotyped retrovirus with a feline immunideficiency virus (FIV) core and an Ebola glycoprotein containing a deletion of codons 309 to 489 in SEQ ID NO:1. At page 22, lines 1-5, the specification states that the

"deletion of amino acids 309-489 from the Ebola glycoprotein (EBOΔO) resulted in a marked 74-fold increase in titer over the average titer obtained with the wild-type Ebola glycoprotein."

Applicants recognize that, in response to a species election requirement made by the Office in an Office Action mailed October 31, 2007, retroviral core and control elements from the species Mo-MuLV were elected. However, Applicants note that examination has been extended to the genus of retrovirus core and core elements, and not confined to Mo-MuLV. Thus, Applicant submits that the disclosure in the specification of an FIV retroviral core pseudotyped with an Ebola glycoprotein containing a deletion of an amino acid sequence encoded by codons 309 to 489 in SEQ ID NO:1 is highly relevant to the written description rejection.

Applicants respectfully submit that possession of the claimed genus has been demonstrated by comprehensively describing, as working examples, these two very different pseudotyped retroviruses. These two retroviruses are representative of the two main types of human retrovirus. Mo-MuLV is an oncoretrovirus, which does not generally transduce non-dividing cells. FIV, on the other hand, is an example of a lentivirus, which is characterized by the ability to transduce non-dividing cells. See Sanders, Expert Opinion Biol. Ther. (2004) 4(3):329-336, 330, first column (IDS mailed November 26, 2007; also the specification at page 10, lines 13-15). Applicants further point to Manicassamy et al., J. Virol. (2005) 79:4793-4805 (included on an Information Disclosure Statement submitted July 22, 2009), which cites extensively to Applicants' publication (Jeffers et al., J. Virol. (2002) 76:12463-12472, cited on an Information Disclosure mailed November 21, 2007; see Example I of the specification), wherein human immunodeficiency virus (HIV) is also successfully pseudotyped with a similar Ebola GP1 deletion mutant (Δ309-476). FIV, HIV and other lentiviruses are described in the specification at, for example, page 10, lines 8-13. Thus, Applicant submits the specification describes examples that utilize, as a retroviral core, not only a simple retrovirus such as a murine leukemia virus (MuLV), but also a more complicated retrovirus such as a lentivirus, such as feline

immunodeficiency virus (FIV) and human immununodeficiency virus (HIV) (see specification at page 10, lines 8 to 19).

At paragraph 15 of the Office Action mailed April 1, 2009, the Office further asserts that there is unpredictability in the performance of the species such that disclosure of a single species does not place Applicants in possession of the genus. Brindley et al. (J. Virol. (2007) 81:7702-7709 is post-filing art cited by the Office as teaching the unpredictability of the performance of a retrovirus pseudotyped with a glycoprotein comprising a modified *O*-glycosylation region.

However, as noted above, the claims have been amended to recite a specific glycoprotein, i.e., an Ebola glycoprotein containing a deletion of an amino acid sequence encoded by codons 309-489 of SEQ ID NO:1. Brindley et al. use, as their *parental plasmid*, the plasmid described in the instant specification at page 21, line 19: a plasmid containing the Ebola GP gene (EbolaΔO) with a deleted GP1 mucin domain (pEZGPΔ309-489). All other mutations described in Brindley et al. are built on top of this platform, using a site-directed mutation technique known as "alanine scanning mutagenesis." Since claims 1 and 13 have been amended to recite "a deletion of an amino acid sequence encoded by codons 309-489 of SEQ ID NO:1 in the *O*-glycosylation region" and claim 8 depends from claim 1, it is respectfully submitted that any unpredictability of the 63 variants of the Ebola glycoprotein taught in Brindley et al. is not applicable. Indeed, the fact that the mucin domain deletion Ebola GP construct (Δ309-489 GP) was used by Brindley et al. as the *parental construct* for incorporation into FIV virions (Brindley et al. at page 7704, first col.) attests to the reliability of the claimed pseudotyped retrovirus.

Applicants thus respectfully submit that the instant specification supports the scope of claims 1, 8 and 13, as amended herewith. The specification teaches that a retrovirus pseudotyped with an Ebola glycoprotein that comprises a deletion of an amino acid sequence encoded by codons 309 to 489 in SEQ ID NO:1 demonstrates predictably increased transduction efficiency. At least two distinct species of the claimed genus of retroviruses are described, each of which demonstrates at least 2-fold higher transduction efficiency than a retrovirus pseudotyped with the wild-type glycoprotein. Applicants therefore respectfully submit that one of skill in the art would readily conclude that Applicants were indeed in possession of the claimed invention.

Specifically, Applicants were in possession of the genus of retroviruses pseudotyped with an Ebola glycoprotein containing a deletion of codons 309 to 489 in SEQ ID NO:1, as recited in claims 1 and 13, as amended, and in claim 8, as depending from claim 1, that have a transduction efficiency of at least 2-fold higher than a retrovirus pseudotyped with the wild-type glycoprotein.

For at least the reasons set forth above, it is respectfully submitted that claims 1, 8, and 13 meet the written description requirement as set forth in 35 U.S.C. §112, first paragraph. Reconsideration and withdrawal of the rejection of claims 1, 8, and 13, under 35 U.S.C. §112, first paragraph, is accordingly requested.

Rejection under 35 U.S.C. §103(a)

Applicants find paragraphs 15 and 16 of the Office Action mailed November 25, 2009, setting forth the claim rejections under 35 U.S.C. §103(a) to be somewhat confusing as it appears portions of the text of the prior rejection may have been unintentionally omitted or repeated. However, in the interest of advancing prosecution Applicants will proceed on the assumption that the claims presently under examination, namely, claims 1, 2, 6, 8, 9, 11 and 13, are rejected as being unpatentable under 35 U.S.C. §103(a) over Yang et al. (Nature Medicine, 2000, 6(8):886-889, Yang S. (Hum Gene Ther. 1999, Jan 1;10(1):123-132), Simmons et al. (J. Virology, 76(5):2518-2582, 2002), Wool-Lewis (J. Virology, 1998, 72(4):3155-3160) (referred to in the Office Action as "Wood-Lewis") and Soneoka Y et al. (Nucleic Acid Res. 1995, Vol. 23(4):628-633). If this is incorrect, Applicants kindly request that the Examiner remove the finality of the present Office Action and reiterate the rejection in a subsequent non-final Office Action.

Applicants respectfully maintain that present invention, as set forth in the pending claims, is nonobvious over the cited art.

More particularly, Applicants submit that the Office has failed to make out a prima facie case of obviousness of the claimed invention over the cited art. More particularly, Applicants submit that the cited art does not teach or suggest all the elements of the claimed invention, nor does it render obvious the claimed invention as a whole.

Yang et al. (2000) describe a mutant Ebola GP protein, GP(Δmuc), as "a mutant with an internal deletion in a serine-threonine-rich, mucin-like region" (Yang et al. at page 886, second col.).

However, contrary to the Examiner's statement that the deletion comprised amino acids 315-505, Yang et al. do not define their deletion. The deleted region is not identified by any start and stop residues, nor do Yang et al. teach what portion of the Ebola GP sequence is missing. See Sanders (2004) at page 333, second col., which states in reference to Yang et al.: "[a] major problem with the data is that the constructed deletion is not described in the article." The amino acid sequence cited by the Office, amino acids 315-505, relate to a DNA fragment that was *inserted into* envelope protein 4070A of MLV and subcloned in to an *expression* vector (Yang et al. at page 889, second col.); Yang et al. do not reference this sequence in connection with the GP1 *deletion.*

Yang et al. did investigate the "pseudotyping" ability of their deletion mutants, including the undefined GP(Δmuc), using a "green fluorescent protein [GFP] reporter assay." A CMV-MLV (MLV: murine leukemia virus--see Yang et al. (1999)) hybrid retroviral vector expressing GFP was used to generate the pseudotyped retroviral vector. Human endothelial cells were infected with the pseudotyped viruses, and GFP expression was measured. On the basis of this assay (Fig. 1b), Yang et al. concluded that GP(Δmuc) was "expressed and functionally active at levels similar to those of wild-type GP" (Yang et al. at page 886, second col.).

However, Yang et al. does not describe increased titer levels or transduction efficiency for the pseudotyped retroviruses.

Simmons et al. teach a murine leukemia virus (MLV; see Wool-Lewis et al. and Soneoka et al.) pseudotyped with an Ebola GP that contains a deletion in the mucin-like domain of GP1 (Simmons et al., page 2519). Several mutants are taught, including one, mutΔ1234, which has a deletion between amino acid 311 and 463 (Simmons, Fig. 1A) and which lacks all of the predicted C-terminal O-linked glycosylation sites (Simmons et al., page 2520 bridging to page 2521). As in Yang et al., GP expression (measured as GP2 levels) was found to be about the same for the mutants as well as the wild-type GP (see Fig. 1B which shows GP expression in

mutΔ1234 at 136% of wild-type GP). Coexpression of mutΔ1234 with plasmids encoding MLV Gag/Pol and LacZ produced infectious MLV pseutotype particles with titers equivalent to those of wild-type EboZ GP (Simons et al., page 2520 bridging to page 2521).

Simmons et al. do not describe increased transduction efficiency for any of the GP mutants, nor do they teach the claimed Ebola GP mutant, Ebola GP1 deletion mutant (Δ309-489).

Both Yang et al. and Simmons et al. represent attempts to elucidate the determinants of Ebola's vascular cytotoxicity and injury, and both use deletion mutants to explore the effects on cell morphology, cell death, and the like. However, "[i]n neither study was it found that deletion of the mucin domain had a major effect on Ebola GP-pseudotyped virus production" (Sanders (2004) at page 333, second col.; emphasis added).

Unlike Yang et al. and Simmons et al., Applicants have created a pseudotyped retrovirus containing a mutant Ebola GP having a deletion of the mucin domain that *does* result in a major effect on Ebola GP-pseudotyped virus production. Claim 1 is drawn to a pseudotyped retrovirus that includes, *inter alia*, a retroviral core and an Ebola glycoprotein containing a deletion of codons 309-489 of SEQ ID NO:1 in the *O*-glycosylation region. Claim 8, which depends from claim 1, recites a transduction efficiency into target cells of at least 2-fold higher than a retrovirus pseudotyped with the wild-type glycoprotein.

The Declaration of inventor David Sanders, Ph.D., submitted herewith, demonstrates how the present invention significantly advanced the art. In his Declaration, Dr. Sanders cites his publication, Sanders (2004), which reports how the field of gene therapy was advanced by the claimed invention. In describing a novel pseudotyped retrovirus of the invention, Sanders states at page 333:

> Remarkably, deletion of virtually all of this region of *O*-glycosylation resulted in the enhancement of Δ309-489 GP processing and incorporation into recombinant MuLV. Consequently, dramatically higher transduction titres were obtained.

(citing Applicants' publication Jeffers et al. (2002); see also Example I of the specification).

And at Sanders (2004), page 333 bridging to page 334:

> Thus, deletion of the mucin domain . . . enhances transduction by pseudotyped retroviruses. This . . . was confirmed when it was demonstrated that FIV pseudotyped with the Ebola Δ309-489 GP possessed a 74-fold higher transduction titre than FIV pseudotyped with the wild-type Ebola GP

(citing Sinn et al., J. Virol. (2003) 77:5902-6910, submitted with the Information Disclosure Statement mailed on November 21, 2007; see also Example III in the specification).

And further at Sanders (2004) page 334:

> Indeed, it was the Ebola Δ309-489 GP-pseudotyped FIV that was employed in the human airway epithelium transduction experiments described above. The advantages of pseudotyping of lentiviruses (HIV-1 and FIV) with Ebola GP with a deletion of the mucin domain have recently been confirmed"

(citing Medina et al., Mol. Ther. (2003) 8:777-789, submitted on an Information Disclosure Statement mailed November 21, 2007).

The Office concedes that neither Yang et al. nor Simmons et al. explicitly teaches deletion of amino acids 309-489 of Ebola GP, nor a pseudotyped MLV with a modified Ebola GP having transduction efficiency into a target cell of at least 2-fold higher than a retrovirus pseudotyped with the wild-type GP. However, the Office characterizes the selection of the deletion region, Δ309-489, as nothing more than a "design choice," arguing that Yang et al. and Simmons et al. "have shown that pseudotyped MLV with a modified Ebola GP comprising a deletion of either amino acids 315-505 or amino acids 311-467 can be used for gene transfer and with reduced GP toxicity to host cells."

Applicants respectfully disagree with the Office's characterization of the claimed invention as a mere "design choice." The invention was not a simple substitution of one known element for another to obtain predictable results, nor does it represent a choice from a finite number of identified, predictable solutions, with a reasonable expectation of success. As noted in the Declaration of Dr. Sanders, the significantly increased transduction titers associated with

this particular construct were not predictable, and any expectation of success would not have been reasonable. One of skill in the art of gene therapy would not have foreseen any particular benefit in choosing to delete amino acids Δ309-489 from the Ebola glycoprotein, particularly when other deletion mutants had shown no meaningful increase in transduction titer.

The specification states, and the declaration of Dr. Sanders confirms, that deletion of codons 309 to 489 in SEQ ID NO:1 of Ebola glycoprotein led to a striking and unexpected increase in *both* expression of Ebola glycoprotein *and* transduction efficiency of a retrovirus pseudotyped with this glycoprotein. The specification states as follows.

> At page 19, lines 11-12:
>
> Remarkably, processing and viral incorporation of the Δ309-489 GP was greatly enhanced as shown in Figure 2.
>
> At page 19, lines 17-19:
>
> There was also a corresponding increase of 696 ± 142% in transduction by the Δ309-489 GP pseudotyped viruses as compared to wild type.
>
> And at page 19, lines 27-32:
>
> The effect of deleting the *O*-glycosylation region of GP_1 (Δ309-489) on expression and transduction were striking. This segment, which is rich in proline, serine, and threonine residues is the most variable among the Ebola GPs. Elimination of this mucin-like region results in enhanced GP processing and incorporation into retroviral particles (Figure 2) and consequently higher levels of transduction by the pseudotyped retroviruses.

The Office Action states that there is no "factual evidence" in the specification showing how the effect of deleting the O-glycosylation of GP1 (Δ309-489) on expression and transduction were striking, but Applicants respectfully disagree. At Fig. 2, which shows expression and incorporation of the Δ309-489 Ebola GP into pseudotyped retroviruses, the specification provides factual support for the contention that the effect of the modified Ebola GP on *expression* was striking. As noted in the Declaration of Dr. Sanders, it is not the protein *size*

(i.e., migration time) that is relevant in this respect, but the significantly increased *density* (amount) observed for the Δ309-489 modified proteins in Fig. 2. Likewise, with respect to *transduction*, the specification reports the factual results of an experiment stating that "[t]here was also a corresponding increase of 696 ± 142% in transduction by the Δ309-489 GP pseudotyped viruses as compared to wild type. " Additionally, as noted in Dr. Sanders' Declaration, Jeffers et al., Journal of Virology, December 2002, p. 12463-12472, Vol. 76, No. 24, submitted on an Information Disclosure Statement mailed November 21, 2007, which was published by the inventors after filing of the priority document, follows up that statement with the data below in Table 3, showing transduction of NIH 3T3 cells by virus pseudotyped with several different mutant Ebola virus GPs with altered glycosylation:

Mutant GP	% Transduction[a]
N40D	<0.1
T42D	113 ± 17
N204D	102 ± 14
N238Y	88 ± 4
N257D	88 ± 9
N277D	84 ± 10
N296D	62 ± 10
N563D	80 ± 4
N618D	102 ± 3
Δ309-489	696 ± 142

[a]Relative to that for the wild type. The average transduction by virus bearing wild-type GP was 1.4×10^4 transducing units/ml.

Jeffers et al. go on to note that "[t]ransduction by Ebola virus 309-489 GP-pseudotyped viruses is increased from the relatively mediocre titers achieved with wild-type GP to levels that are comparable to those achieved with standard vesicular stomatitis virus G protein-pseudotyped viruses in our system."

For at least the reasons set forth above, we submit that a prima facie case of obviousness has not been made out by the Office, and further that, in any event, the claimed invention yielded

unexpected results. Reconsideration and withdrawal of the rejection of claims 1, 2, 6, 8, 9, 11 and 13, as being unpatentable under 35 U.S.C. §103(a) are respectfully requested.

Request for Rejoinder

Species elections were required by the Examiner in the Office Action dated October 31, 2007. It is understood that (a) the requirement for species election will be withdrawn upon the finding of an allowable genus; and (b) any species withdrawn from consideration will be transferred to the elected subject matter unless it is found patentably distinct from the elected or allowed claims.

Applicants respectfully submit that claims 1, 2, 6, 8, 9, 11 and 13, as amended herewith, are in condition for allowance.

In view thereof, Applicants kindly request withdrawal of the species elections and rejoinder of claims 3, 4, 7, and 12, which currently stand withdrawn. Claims 3, 4 and 7 depend from claim 1, which, as amended, which remains generic. Claim 12, as amended, depends from claim 9, which also remains generic. With respect to the genus of retroviral core and control elements, Applicant wish to draw the Examiner's attention to Example III in the specification, beginning at page 21, which describes a retrovirus with a FIV core and control elements (see, e.g., claims 3, 4 and 12), pseudotyped with an Ebola glycoprotein containing a deletion of amino acids 309-489 in the *O*-glycosylation region as recited in claim 1 (from which claims 3 and 4 depend) and claim 9 (from which claim 12 depends).

Summary

It is respectfully submitted that the pending claims 1, 2, 6, 8, 9, 11 and 13 are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to contact Applicants' Representatives at the telephone number listed below if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted
By
Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581336
Minneapolis, MN 55458-1336
Phone: (612) 305-1220
Facsimile: (612) 305-1228
Customer Number 26813

March 25, 2010
Date

By: David L. Provence
David L. Provence
Reg. No.43,022
Direct Dial (612) 305-305-1005

CERTIFICATE UNDER 37 CFR §1.6:
The undersigned hereby certifies that this paper is being transmitted via the U.S. Patent and Trademark Office electronic filing system in accordance with 37 CFR §1.6(a)(4) to the Patent and Trademark Office addressed to the Commissioner for Patents, Mail Stop RCE, P.O. Box 1450, Alexandria, VA 22313-1450,
on this 25 day of March, 2010.

By: Sandy Truehart
Name: SANDY TRUEHART